

USB® HotStart-IT® SYBR® Green qPCR Master Mix (2X)



- **Complete master mix with SYBR Green**
 - Compatible with standard SYBR filters
 - Multiple platform capability - includes separate tubes of ROX™ and Fluorescein Passive Reference Dyes to allow for normalization on different instruments
- **Based on novel Hot Start method of primer sequestration**
 - No DNA template damage - no extensive heating step needed to denature hotstart component
 - Avoids non-specific products and primer-dimer formation
 - Room temperature reaction set-up
- **Highest sensitivity with broad dynamic range**
 - Detects fewer than 10 target copies
 - Performs over a linear dynamic range of 7 to 8 orders of magnitude with a minimal correlation coefficient = 0.95

DNA Polymerase, MgCl₂, Ultrapure nucleotides, and SYBR Green I for use in real-time quantitative PCR reactions (qPCR). Simply add DNA template, primers, and water and the reactions are ready for cycling. Separate tubes of the passive reference dyes, ROX (for ABI and Stratagene instruments) and fluorescein, (for BioRad instruments) are included for added convenience.

The master mix has SYBR Green I dye which detects any double-stranded DNA that accumulates during the amplification process. The hot start feature enhances SYBR-based qPCR reactions by reducing primer-dimer formation which increases specificity and sensitivity. HotStart-IT SYBR Green qPCR Master Mix has excellent sensitivity as it detects fewer than 10 target copies, performs over a broad, linear dynamic range of 7 to 8 orders of magnitude, and is compatible with a variety of real-time PCR instruments. The mix does not have dUTP in place of dTTP and is incompatible with carry-over contamination prevention methods using Uracil-DNA Glycosylase. For carry-over prevention methods, use HotStart-IT SYBR Green PCR Master Mix with UDG (2X), PN 75760.

USB HotStart-IT SYBR Green qPCR Master Mix uses a novel hot start method designed and developed at Affymetrix called primer sequestration. With this method, a protein binds and sequesters primers at lower temperatures making them unavailable for use by Taq DNA Polymerase. Following the initial denaturation step, the protein is inactivated and the primers are released (Fig. 1). HotStart-IT SYBR Green qPCR Master Mix is supplied as a 2X pre-mixed formulation containing HotStart-IT Taq

Convenient

For a 50 µl reaction, simply add 25 µl of master mix to primers, DNA template and PCR-Qualified H₂O. Reactions can be tailored from 20 µl to 100 µl volumes. Room temperature reaction assembly is possible because of the hot start feature.

Fig. 1. USB HotStart-IT method: primer sequestration

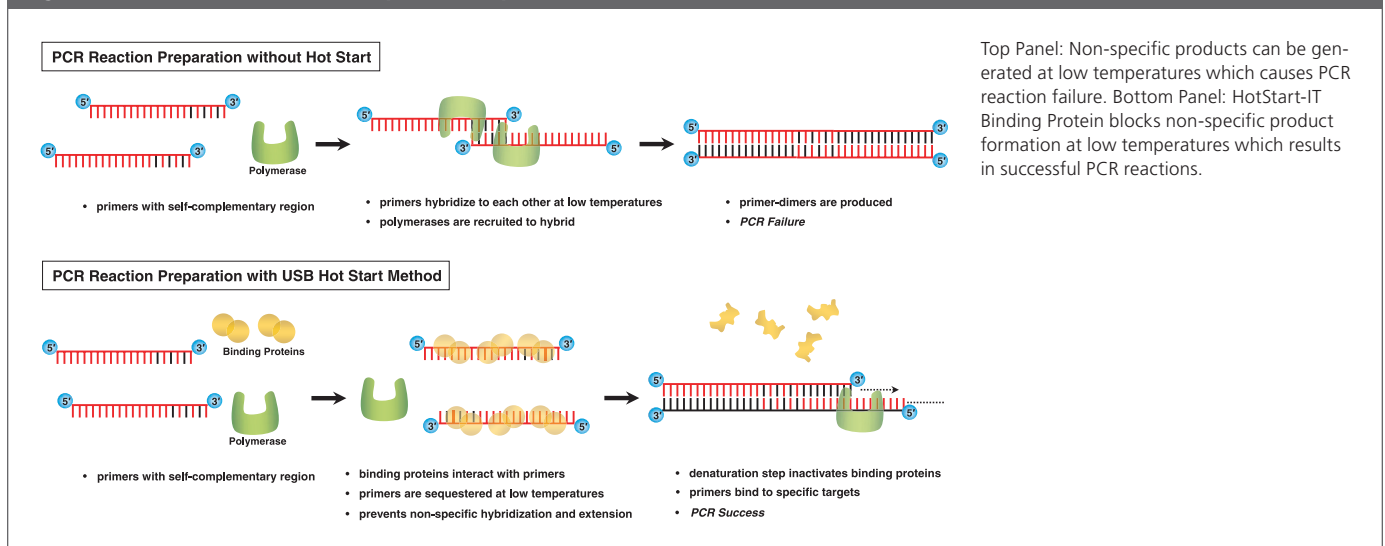
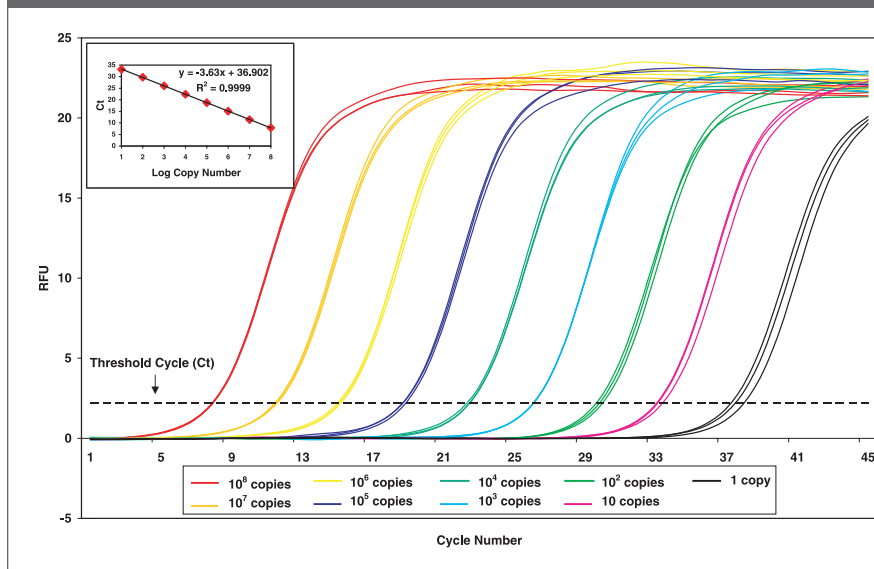


Fig. 2. Real-time PCR amplification



GAPDH Assay using HotStart-IT SYBR Green qPCR Master Mix (PN 75762). Triplicate reactions were performed with a cloned region of the human GAPDH gene as template using an ABI 7500 Fast instrument. The non-specific dsDNA binding dye, SYBR Green I, was used to detect the 122 bp amplicon and ROX was used as a passive reference dye. The amplification process was linear over eight orders of magnitude (see inset) and a single copy of the target could be efficiently detected. The No Template Control (NTC) reaction generated no measurable fluorescence.

Novel hot start technology

The mix does not use Taq antibodies which eliminates potential mammalian-source DNA contamination. Also, since the polymerase is not chemically-inactivated, no extensive initial heat-activation step is necessary which reduces damage to precious DNA samples.

Higher specificity, sensitivity and broad dynamic range

The hot start feature minimizes amplification of non-specific products and primer-dimers. The SYBR Green I concentration has been carefully optimized for maximum sensitivity and can be used in melt-curve analyses. PCR products are amplified with low background and from low-copy targets with a linear dynamic range of 7 to 8 orders of magnitude (Fig. 2).

Stable

Repeated freeze-thaw cycles have no observed effect on performance.

Tested User Friendly™ functional test:

Real-time PCR reactions were performed on an ABI 7500 Fast Instrument using primers specific to a 122 bp cloned region of the human GAPDH gene as template. Product specifications require that the correlation coefficient from a linear regression over seven orders of magnitude (10 to 10⁷ template copies) must be greater than or equal to 0.95.

For research use only. Not for use in diagnostic procedures.

Affymetrix, USB and HotStart-IT are registered trademarks of Affymetrix, Inc. Tested User Friendly is a trademark of Affymetrix, Inc. HotStart-IT Taq DNA Polymerase—Methods for using this product may be covered by US Patent No. 7,700,281. Taq DNA Polymerase—sold under licensing arrangements with Applied Biosystems. Purchase is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Perkin-Elmer or as purchased, i.e., an authorized thermal cycler. SYBR is a registered trademark of Molecular Probes, Inc. and is provided under an agreement with Molecular Probes, Inc. ROX is a trademark of Applera Corporation or its subsidiaries in the US and certain other countries.

HotStart-IT SYBR Green qPCR Master Mix (2X):

The mix combines USB HotStart-IT Taq DNA Polymerase, SYBR Green I, MgCl₂, and Ultrapure nucleotides in a unique buffer formulation. Magnesium and nucleotide concentrations are 5 mM and 0.4 mM each, respectively.

Components:

HotStart-IT SYBR Green qPCR Master Mix (2X):
 100 reactions (2 x 1.25 ml)
 500 reactions (12.5 ml)
 25 mM MgCl₂
 ROX Passive Reference Dye
 Fluorescein Passive Reference Dye
 Brief protocol

Shipping and storage:

Shipped on dry ice. Store at -20°C. Mix well prior to use.

HotStart-IT SYBR Green qPCR Master Mix (2X)

Product code	Pack size
75762	100 reactions
	500 reactions

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